


The glaucomas are a leading cause of blindness globally, and their prevalence is increasing as the population ages. Primary open-angle glaucoma (POAG) is the most common form of the disease, although in some regions of Asia angle closure glaucoma (ACG) is more prevalent. POAG is predominantly composed of high-tension glaucoma (HTG), where intraocular pressure (IOP) is “raised” (IOP >21 mmHg). Normal-tension glaucoma (NTG), which is another important subgroup of POAG, is an optic neuropathy similar to HTG, in which IOP levels are within the statistically normal range (IOP ≤21 mmHg). Lowering IOP reduces the risk of disease.
progression’ and controlling IOP with topical eye drops continues to be the most common first line therapy, for both high- and normal-tension glaucoma. The most commonly prescribed drugs act on either the ciliary process epithelium to reduce aqueous humor production (beta-adrenergic receptor antagonists) or the ciliary muscle cells to increase uveoscleral outflow (prostaglandin analogs that bind to and activate prostaglandin F (FP) receptors). The goal of treatment for all forms of glaucoma is the preservation of visual function.

IOP elevation results from increased outflow resistance in the tissues of the outflow pathways: the trabecular outflow tract (trabecular meshwork or TM) and the uveoscleral pathway. In normal eyes, IOP builds up, in response to the inflow of aqueous humor, to the level sufficient to drive fluid across that resistance at the same rate it is produced by the ciliary body; this is the steady-state IOP. In the study of aqueous humor dynamics an important distinction to make is between aqueous outflow rate (measured in µl/min) and outflow facility (measured as µl/min/mmHg), which refers to hydraulic conductivity, the reciprocal of resistance. In most glaucomatous eyes, the resistance is unusually high, elevating IOP. Flow from the anterior chamber across the TM into Schlemm’s canal is pressure dependent, but drainage through the uveoscleral pathway is essentially independent of pressure at IOP levels greater than 7 to 10 mmHg. In young humans (and young monkeys) the percent flow through each pathway may be close to 50%; with age, TM outflow predominates as uveoscleral outflow declines. Resistance to outflow is thought to be greatest in the juxta-canalicular (JCT) region and inner wall of Schlemm’s canal (SC) (Figure 1) and is directly affected by contraction and relaxation of the ciliary muscle (CM) and TM. Contraction of the CM increases intercellular spaces in the TM, increasing the amount of fluid drained through that pathway. When the CM relaxes, the TM flow pathways become narrower and more resistive while the spaces between the muscle bundles are expanded and uveoscleral outflow is increased. Contractility of the TM also modulates outflow. In perfused anterior eye segments, substances that contract trabecular cells decrease outflow facility, whereas substances that induce relaxation widen intertrabecular flow spaces, increasing outflow facility. Studies of the structural and functional biology of outflow through the TM / SC indicate that many biological pathways intersect and interact to regulate IOP including cholinergic, adrenergic, prostaglandin, cytoskeletal, extracellular matrix (ECM) synthesis and degradation and cell junctional mechanisms.

Recent studies with endothelial nitric oxide synthase (eNOS) knockout mice demonstrate that nitric oxide (NO) serves a biochemical / molecular signaling role in increasing TM (conventional, pressure-dependent) outflow facility; that NO-mediated facility can be manipulated both genetically and pharmacologically and that the NO-mediated facility increase may be triggered by mechanical distension of the TM (Fig-
outflow facility and IOP. The active role of TM contraction and relaxation in the regulation of IOP results from the aggregate of the actomyosin contractility/cytoskeleton/cell–cell and cell–matrix adhesion responses of the individual TM/JCT/SC cells. The cytoskeleton and contractility mechanisms may be the efferent “implementation” arm of the reflexive and regulatory mechanism, governing the final facility. The eNOS/NO system may be a signal/transduction system noted above, modulated by sensors in the CM tendons, the CM apex, the scleral spur and the TM (Figure 3). Taken together the evidence supports the TM being a responsive self-aware, self-regulating tissue/organ.

It may well be possible to manipulate both the afferent and efferent mechanisms that influence the TM cytoskeleton, making it an attractive target for therapeutics aimed at enhancing outflow. Relaxing TM, JCT and SC inner wall cells leads to a tissue configuration that may be a geometrically and biomechanically critical event and a fundamental endogenous control mechanism for outflow resistance. Several classes of compounds act on the TM to disrupt the actin cytoskeleton, altering cell shape, contractility and adhesions, and reducing outflow resistance through the relaxation and expansion of the TM (Figure 4). Rho kinase modulation of aqueous outflow is a therapeutic approach in development by a number of groups, but there are other ways to target manipulation of TM architecture for glaucoma therapeutic purposes. The broad-spectrum protein kinase inhibitor H-7 blocks cellular actomyosin-driven contractility, via inhibition of myosin light chain kinase, rho kinase, or both; overexpression of non-muscle caldesmon uncouples actin from myosin; cytochalasins (fungal metabolites) and latrunculins (sea sponge metabolites), inhibit actin polymerization by different mechanisms (Figure 5); all are potent ocular hypotensive agents and increase trabecular meshwork outflow facility in eyes of nonhuman primates. Derivatives of some of these agents are in human clinical trials and are expected to be complementary with prostaglandins.

Recent studies indicate that TM outflow is also affected by TGF-β2 and consequent downstream mediators that have effects on the ECM, including connective tissue growth factor (CTGF), bone morphogenic protein (BMP), gremlin and the Smad-signaling pathway. TGF-β2 has been implicated in the pathogenesis of POAG, based on elevated levels in the aqueous humor of glaucoma patients and its ability to induce ECM remodeling (collagen formation, ECM synthesis, tissue stiffening) in the TM, which leads to an increase of aqueous humor outflow resistance. In perfused human cultured anterior eye segments TGF-β2 increases ECM deposition in the TM and elevates IOP. CTGF is a TGF-β2 target gene with high constitutive TM expression. Treatment of human TM cells with recombinant CTGF causes distinct changes in gene expression indicating that CTGF is a mediator of the effects of TGF-β2 on ECM synthesis in human TM cells. Actin stress fibers and contractility are

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**Figure 3.** Schematic drawing of the cribri-form elastic-like plexus (CP) connected to the inner wall endothelium (E) of Schlemm’s canal by connecting fibrils (CF). The outer tendons of the CM, insert into the CP so that muscle contraction can influence the aqueous humor pathways through the cribri-form region and the giant vacuoles of the endothelium (both black) into Schlemm’s canal. Arrow indicates footlike connection between endothelial and subendothelial trabecular cell. With permission from Gabelt BT and Kaufman PL, Changes in aqueous humor dynamics with age and glaucoma, Progress in Retinal and Eye Research 24 (2005) 612–637.

**Figure 4.** Light micrographs of trabecular meshwork (TM) and Schlemm’s canal (SC) in monkey eyes treated with 300 µM intracameral H-7 (B) or vehicle (A). The JCT and intercellular spaces are extended following H-7 (arrow in A). Inner wall endothelial cells in H-7-treated eyes are thinner than in controls. SC is dilated in H-7 treated eyes. Inner uveal TM (b) is not significantly affected. Bars are 50 µm. With permission from: Overby, D.R., Stamer, W.D., Johnson, M, The Changing Wall Endothelium, Exp Eye Res. 2009 April; 88(4):656–670. Panels originally published in “H-7 effects on the structure and fluid conductance of monkey trabecular meshwork”. Sabanay I, Gabelt BT, Tian B, Kaufman PL, Geiger B, Arch Ophthalmol. 2000 Jul; 118(7):955–62.
also induced by CTGF in cultured TM cells. The BMP, gremlin and Smad-signaling pathway also plays a role in TGF-β2 induced ECM synthesis in the TM. TGF-β2 and BMP4 act in concert to maintain a balance between ECM deposition and degradation. The BMP antagonist gremlin inhibits BMP-4 activity in cultured TM cells and increases outflow resistance in a perfusion cultured human eye anterior segment model. Gremlin employs canonical TGF-β2/Smad signaling to induce ECM genes and proteins in cultured human TM cells. Gremlin also induces both TGF-β2 and CTGF, which can act downstream to mediate ECM changes in TM cells.

Secreted frizzled-related protein 1 (sFPR-1), an antagonist of the Wnt signaling pathway, is differentially expressed in glaucomatous human TM cells compared with normal human TM cells. Addition of recombinant sFPR-1 to ex vivo perfusion-cultured human eyes decreases outflow facility. Intravitreal injection of an adenosinergic vector encoding sFPR1 in mice produces a titer-dependent increase in IOP. These data indicate that Wnt signaling plays a role in regulating IOP, that increased expression of sFPR1 in the TM appears to be associated with elevated IOP and that inhibiting Wnt signaling is a viable strategy for developing a model of experimental glaucoma.

Adenosine agonists Adenosine A1 and A2a receptor agonists are in development as IOP-lowering therapies. In bovine organ-cultured anterior segments, the adenosine A1 agonist cytochalasin A2 produces an outflow facility increase associated with MMP activation in the TM outflow pathway. In a Phase 1/2 single oculus dose clinical trial, the selective adenosine A1 agonist 89675 significantly reduced IOP in glaucoma patients, reportedly by increasing outflow of aqueous humor through the trabecular meshwork. An adenosine A2a receptor agonist (OPA-6566) is also thought to lower intraocular pressure via an adenosine A2a receptor mechanisms to enhance aqueous humor outflow via the trabecular meshwork. In a Phase 1/2 single oculus dose clinical trial, the selective adenosine A1 agonist 89675 significantly reduced IOP in glaucoma patients, reportedly by increasing outflow of aqueous humor through the trabecular meshwork. An adenosine A2a receptor agonist (OPA-6566) is also thought to lower intraocular pressure via an adenosine A2a receptor mechanisms to enhance aqueous humor outflow via the trabecular meshwork. Novel therapeutic targets will continue to be in demand, as glaucoma is a lifelong condition requiring a multi-faceted, additive approach to medical treatment. Over time, most glaucoma patients will be prescribed multiple topical drops, of varying classes/mechanisms, to control their IOP. Understanding how the outflow system functions is critical for determining how to effectively and efficiently manipulate elements of the system to therapeutic effect.

References

Figure 5. Pathways targeting actomyosin contractility to enhance aqueous humor outflow through the TM. MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; MLC, myosin light chain. With permission from Gabelt BT and Kaufman PL. Changes in aqueous humor dynamics with age and glaucoma, Progress in Retinal and Eye Research 24 (2005) 612–637.